J. Clin. Chem. Clin. Biochem.
Vol. 17, 1979, pp. 537–539

Platelet Counting with a Laser Nephelometer

By *D. J. Giannitsis*

Abt. f. Klin. Haemostaseologie und Transfusionsmedizin in den Universitätskliniken, Homburg/Saar

(Received April 19/October 25, 1978)

Summary: A method for platelet counting is described, based on the Laser nephelometric principle. Experimental results are reported, together with the practical considerations for the standardisation and correlation of the method, and for application of the method in the routine biochemical laboratory.

Venous blood, taken with EDTA-NaCl solution according to *Schulz* et al (1971, Z. Klin. Chem. Klin. Biochem. *9*, 329–333) is used. The blood is centrifuged at 100 *g* for 10 min and 10 µl of the supernatant is added to 3000 µl of a suspending medium (dilution 1:80); 300 µl platelet suspension are read in a nephelometer cuvet or tube against blank. The number of platelets per liter blood are determined with the aid of a standard curve. The sensitivity and reproducibility of the method, and the correlation with the "electronic coulter counting" method are satisfactory.

Thrombocyten-Zählung mit einem Laser-Nephelometer

Zusammenfassung: Es wird eine Methode zur Bestimmung der Blutplättchenzahl beschrieben. Die Methode basiert auf dem Laser-nephelometrischen Prinzip. Experimentelle Ergebnisse werden mitgeteilt, wobei neben der Standardisierung der Methode vor allem praktische Gesichtspunkte, z.B. Korrelation mit anderen Methoden usw., berücksichtigt werden. Die Standard-Methode wird wie folgt angegeben: Venenblut wird mit EDTA-NaCl-Lösung nach der Methode von *Schulz* et al (1971), Z. Klin. Chem. Klin. Biochem. *9*, 329–333) aufgezogen. Anschließend wird das Blut bei 100 *g* 10 min zentrifugiert, 10 µl des Plasma-Überstandes in 3000 µl Suspensionslösung pipettiert (Verdünnung 1:80), und davon werden 300 µl in eine spezielle Küvette oder Röhrchen gebracht. Die nephelometrische Messung erfolgt gegen die Blindprobe (plättchenfreies Plasma), und die relative Lichtstreuung in mV entspricht der Thrombocytenzahl, die dann in einer Standardkurve abgelesen werden kann.

Die Empfindlichkeit und die Reproduzierbarkeit der Methode wie auch die Korrelation zu den Methoden des "Electronic Coulter counter" sind befriedigend.

Introduction

A number of methods of platelet counting in biomedical research as well as in routine laboratories are reported in the literature (1–3). The method of platelet counting with the Coulter (3) principle has now been in use for some time, and recently *Schulz* et al (1) have demonstrated a nephelometric method for platelet counting. The advantages of the nephelometric procedure have also been discussed recently (1).

Here we report a method for platelet counting by means of Laser nephelometry, together with the optimal condition for its operation. The results are correlated with those of other methods of platelet counting. The findings and the possibility for application of our method in biochemical laboratories are briefly discussed.

Material and Methods

Venous blood, taken with EDTA-NaCl solution according to the method described by *Schulz* et al (1) is used as follows for platelet counting: 1.6 ml venous blood with 0.4 ml EDTA (Titriplex III, Merck 0.075 g/l)-dextran (Dextran 150, Pharmacia, Uppsala 0.125 g/l)-NaCl (0.075 g/l)-solution is drawn into a special 2 ml syringe. The contents of the syringe are mixed, and transfered into a centrifuge tube. The blood is then centrifuged at 100 *g* for 10 min and 10 µl of the supernatant (platelet rich plasma) is added to 3000 µl of a suspending medium. 300 µl of the platelet suspension are placed in a 1000 µl nephelometric cuvet and read against a blank (=plasma without platelets). The number of platelets per liter blood are read from a present standard curve.

The suspending medium

Alternative suspending media are 8.5 g/l NaCl solution, 220 g/l albumin solution (Firma Merz & Dade), or a mixture of Ficoll-Hypaque or Ficoll-Urografin solution, specific gravity 1.070,

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preformed as described elsewhere (4). *The standard curve* is derived from a dilution series of platelet stock suspension, obtained from healthy donor, calculated by the electronic Coulter counting method. The nephelometric readings in mV were compared to the known number of platelets.

Laser nephelometric counting is performed using a "Behring"-Nephelometer. *Statistical analysis* is performed on the basis of the correlation between Laser nephelometry and electronic Coulter counting.

Results

The sedimentation of platelets over long periods of time under gravity depends both on density and viscosity of the suspending medium. Figure 1 shows the time-dependent stability of platelets after standing in various media. It becomes obvious that the platelets remain stable in suspended form in a medium of Ficoll-Urografin or Ficoll-Hypaque specific gravity 1.070, or albumin solution (220 g/l), as well as in autologous plasma. Laser nephelometric reading and electronic Coulter-counting of platelets are depicted in figure 2. The correlation between

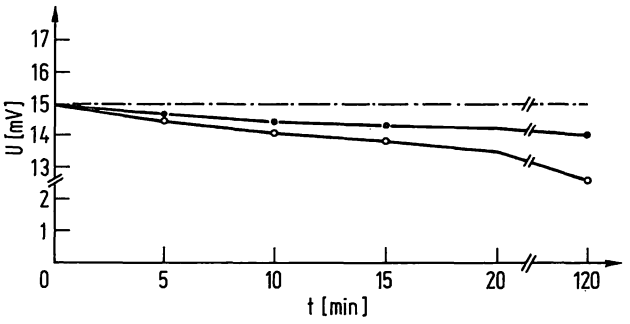

Fig. 1. Curve of time dependent stability (in mV) after standing platelets (samples: n = 10) in various media (normal saline o—o, autologous plasma ●—● and Ficoll-Urografin ·—·—· solution).

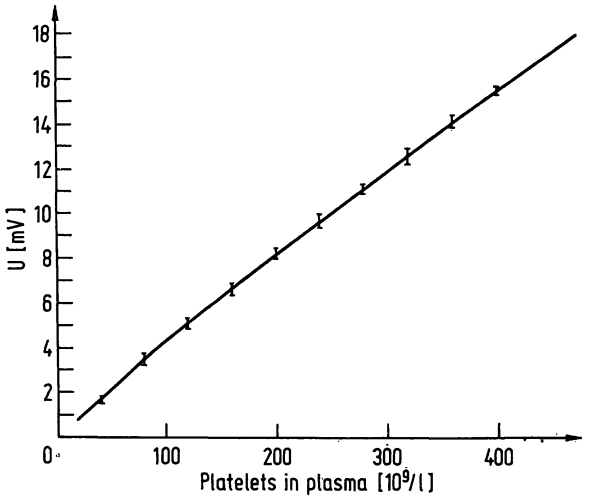

Fig. 2. Correlation between "electronic coulter" counting of platelets and Laser nephelometric principle. The number of platelets after an "electronic coulter" counting (horizontal coordinate) was correlated to the Laser nephelometric light scattering in mV (vertical coordinate; $\bar{x} \pm s$).

Laser nephelometry and electronic Coulter counting is shown in table 1. The sensitivity of the method, depending on the concentration of the suspended particles, was studied against a standard amount of platelets in various concentrations. The dilution is performed with normal saline.

Tab. I. The relationship between electronic Coulter counting and the Laser nephelometric method for platelet counting, (Electronic Coulter counting = X, Laser nephelometric = Y, n = 56; The correlation coeff. (r) = 0.89).

Blood-sample Nr.	Electronic Coulter [10^9/l]	Laser Nephelometer [10^9/l]
1	193	250
2	285	300
3	218	180
4	85	75
5	77	70
6	133	185
7	157	160
8	331	350
9	170	200
10	178	180
11	45	60
12	210	265
13	174	183
14	412	460
15	121	130
16	157	98
17	189	190
18	153	145
19	21	20
20	102	85
21	14	12
22	267	210
23	260	250
24	128	150
25	64	62
26	108	98
27	213	245
28	369	380
29	256	240
30	402	450
31	290	295
32	320	360
33	12	11
34	234	180
35	118	100
36	290	320
37	249	235
38	87	85
39	310	305
40	231	271
41	294	250
42	252	250
43	220	221
44	298	300
45	121	115
46	187	201
47	236	250
48	48	55
49	299	280
50	277	260
51	345	350
52	192	164
53	265	283
54	379	400
55	327	120
56	65	65

Tab. 2. The reproducibility of platelet counting (n = 10) by Laser nephelometry, and the standard deviations.

Platelets count $\bar{x}$ [10^6/l]	± s [10^6/l]
0.40	0.00022
0.80	0.00046
1.20	0.00060
1.60	0.00081
2.00	0.00099
2.40	0.00128
2.80	0.00128
3.20	0.00137
3.60	0.00150
4.00	0.00268

The reproducibility was checked for the same samples (N=10) and the standard deviation is also shown in table 2. The statistical relationship and correlation between both methods, Laser nephelometry and electronic Coulter counting, is represented in table 1.

Discussion

The advantages of the use of the nephelometric counting of platelets in comparison to other methods is discussed elsewhere (*Schulz* et al., (1)). Laser nephelometric measurement follows the law of *Stockes* (1) as well as the equation: $I = \frac{C}{K}$ (I = scattered light; C = concentration of platelets, K = instrumental and working conditions (5)).

A problem of blood cell counting by nephelometric means lies in the rate of sedimentation of the particles.

Other problems are reproducibility of measurement, precision of the method and correlation with other methods. From figure 1 can be seen that the sedimentation rate of platelets in the first 20 minutes is very small, and the difference in count is less than 8% when using autologous serum, compared to other media like Ficoll-Hypaque, etc.

This is verified by the standard deviation (tab. 2) when counting the same platelet preparations. Precision of the method depends on the number of particles, as shown in table 1 and figure 2. The correlation coefficient between Laser nephelometry and electronic Coulter counting is also presented in table 1 and figure 2.

The method of Laser nephelometry should be well suited for application in clinical laboratories, and may be combined with measurements of other cells or substances; also the method could be mechanized.

Acknowledgement

I thank Mr. *R. Fuchs* for technical assistance.

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Dr. D. J. Giannitsis
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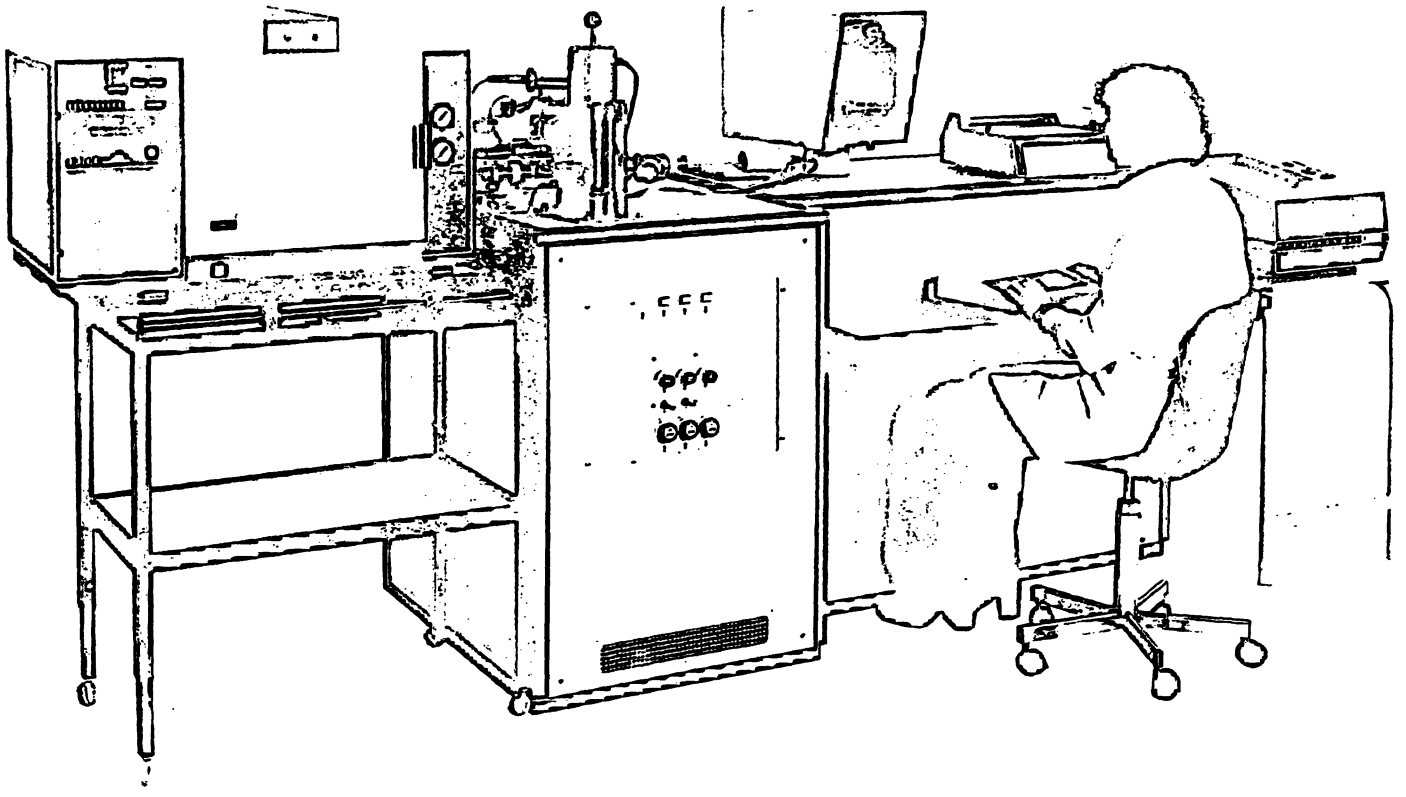

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